Vestnik zoologii, **48**(3): 203–210, 2014
DOI 10.2478/vzoo-2014-0023

UDC 595.132:595.5:502.72(477)

FINDINGS OF ENTOMOPATHOGENIC NEMATODES (RHABDITIDA, STEINERNEMATIDAE) IN NATURE RESERVES IN UKRAINE

Ye. B. Yakovlev[1], V. A. Kharchenko[1], Z. Mráček [2]

[1]*Schmalhausen Institute of Zoology, NAS of Ukraine,*
vul. B. Khmelnytskogo, 15, Kyiv, 01601 Ukraine
[2]*Institute of Entomology, Biology Centre CAS,*
Branišovská, 31, České Budějovice, 370 05 Czech Republic

Findings of Entomopathogenic Nematodes (Rhabditida, Steinernematidae) in Nature Reserves in-Ukraine. Yakovlev, Ye. B., Kharchenko, V. A., Mráček, Z. — Five strains of *Steinernema* Travassos, 1927 were isolated by live baiting method with last instar larvae of *Tenebrio molitor* Linnaeus, 1758 from the reserves of some central and southern oblasts of Ukraine and the Crimean AR. Entomopathogenic nematodes were recovered from 5 of 196 (2.6 %) soil samples collected in 2010. Isolated nematodes were identified using a combination of molecular (ITS1–5.8S–ITS2 rDNA gene sequencing) and morphological techniques. Four of the isolated strains were recognized as *S. feltiae* (Filipjev, 1934), one as *S. arenarium* (Artyukhovsky, 1967).

K e y w o r d s: entomopathogenic nematodes, *Steinernema*, nature reserves.

Находки энтомопатогенных нематод (Rhabditida, Steinernematidae) в природных заповедниках Украины. Яковлев Е. Б., Харченко В. А., Мрачек З. — Пять штаммов *Steinernema* Travassos, 1927 было получено из заповедников некоторых областей центра и юга Украины, а также Крымской АР методом живых ловушек с применением личинок *Tenebrio molitor* Linnaeus, 1758 последнего возраста. Энтомопатогенные нематоды были выделены из 5 среди 196 (2,6 %) почвенных проб, собранных в 2010 г. Выделенные нематоды идентифицированы комбинацией молекулярного (секвенирования гена ITS1–5.8S–ITS2 рДНК) и морфологического методов. Четыре из выделенных штаммов определены как *S. feltiae* (Filipjev, 1934), один как *S. arenarium* (Artyukhovsky, 1967).

К л ю ч е в ы е с л о в а: энтомопатогенные нематоды, *Steinernema*, природные заповедники.

Introduction

Entomopathogenic nematodes (EPN) of the families Steinernematidae and Heterorhabditidae are important agents for biological control of pest insects (Erbaş et al., 2014; Hominick, 1990; Laznik et al., 2010; Laznik et al., 2011). They are widespread in different biogeographic zones, excluding the Arctic and Antarctica. There are more than 100 species known in both families. EPN have wide range of arthropod hosts; they infect species from all the orders of Insecta, some Isopoda (such as *Armadillum officinalis* Latreille, 1804) (Sicard et al., 2008) and mites (*Ixodes ricinus* L., 1758) (Hartelt et al., 2008). EPN's collecting and investigations is important for finding new highly pathogenic strains adapted for different conditions and their using as biological control agents (Yan et al., 2012; Negrisoli et al., 2013; Laznik, Trdan, 2013).

First EPN species, *Steinernema kraussei* (Steiner, 1923) Travassos, 1927 was described by Steiner in 1923 from *Lyda* sp. sawfly larvae (Steiner, 1923) as a new species of the genus *Aplectana* Railliet et Henry, 1916. I. N. Filipjev (1934) estabilished the new family Steinernematidae. G. O. Poinar (1976) described the family Heterorhabditidae, and since that period the number of species in both families has extremely arisen to more than one hundred species.

During three decades (from1950 till1970), entomopathogenic nematodes were studied in the agricultural nematology in the European part of the Soviet Union (Ukraine, Byelorussia and Leningrad Region of Russia) (Kiryanova and Puchkova, 1955, Veremchuk, 1969). Four species of *Neoaplectana* Steiner, 1923 were described from different hosts and later considered as species inquirenda or as subspecies of *Steinernema feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin et Bedding, 1982 or *S. carpocapsae* (Weiser, 1955) Wouts, Mráček, Gerdin et Bedding, 1982 (Nguyen, Hunt, 2007).

Material and methods

Collecting and isolation of entomopathogenic nematodes. Soil samples have been collected in the nature reserves located in Dnipropetrovsk, Donetsk and Kherson oblasts and in the Crimea during the field season of 2010 (fig. 1). They were taken at 5–15 cm depth in volume of 350 ml. Totally, 196 soil samples from 7 reserves were collected.

Abiotic factors of sampling sites, such as temperature and humidity were reconstructed from the archive data of Meteopost website (http://meteopost.com). They were the following: Dniprovsko-Orilsky Nature Reserve — 38 °C, 18 %; Chornomorsky Biosphere Reserve, Ivano-Rybalchansky Plot — 38 °C, 24 %; Ukrainian Steppe Nature Reserve, "Kamyani Mohyly" — 39 °C, 15 %; Karadag Nature Reserve — 30 °C, 36 % (according to nearest to the Nature Reserve city, at 15.00 p. m.).

Entomopathogenic nematodes were isolated from the soil in laboratory by the baiting method with *Tenebrio molitor* Linnaeus, 1758. Soil samples were kept in paper bags with the last (IX–XII) instar larvae of *T. molitor*. After the 5 days incubation, dead larvae were removed and replaced by the new batch of living larvae. This procedure was repeated four times. Dead host larvae were individually incubated on the White's traps until the new generation of infective juveniles (IJ) migrated to the water trap (White, 1927).

Morphological and morphometric research

Nematode observation. Nematode isolates were studied morphologically and morphometrically. The nematodes studied were heat killed in 50–60 °C TAF-solution and kept in the fixative for 1 day. After fixation, nematodes were put into anhydrous glycerol according to Seinhorst (1959) method and mounted on permanent slides. Observations and measurements were done on Axio Imager M1 Carl Zeiss™ microscope with DIC optics at ×10 and ×40 magnifications.

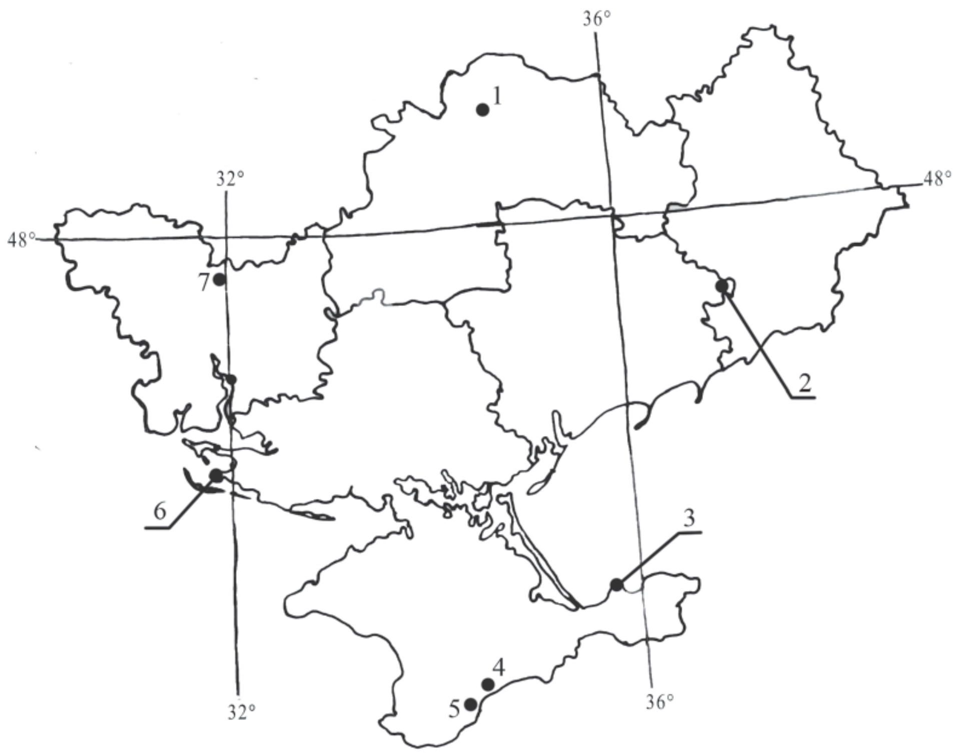


Fig. 1. Map of Ukraine with marked areas of soil sampling: 1 — Dniprovsko-Orilsky Nature Reserve; 2 — Ukrainian Steppe Nature Reserve, "Kamyani Mohyly"; 3 — Kazantip Nature Reserve; 4 — Karadag Nature Reserve; 5 — Crimean Nature Reserve; 6 — Chornomorsky Biosphere Reserve, Ivano-Rybalchansky District; 7 — Nature Reserve "Yelanetsky steppe".

Рис. 1. Карта Украины с указанными точками отбора почвы: 1 — Днепровско-Орельский природный заповедник; 2 —Украинский степной природный заповедник «Каменные могилы»; 3 — Казантипский природный заповедник; 4 — Карадагский природный заповедник; 5 — Крымский природный заповедник; 6 — Черноморский биосферный заповедник, Ивано-Рыбальчанский участок; 7 — природный заповедник «Еланецкая степь».

```
                             ....|....|....|....|....|....|....|....|....|....|....|....|
                                 5        15        25        35        45        55
S. arenarium isolate IR      ------------------------------------------------------------
S. feltiae isolate KM        -----------CCAGACTAGAACTTTCCTCCGCTAACTGATATGCTTAAGTTCAGCGGGT
S. feltiae isolate Kar-13    --GGGGGGGGCATCGGCCC-GGCGGGGCGGACACGCGAAAGAAGGGAAGTTCAAAAAGGT
S. feltiae isolate Kar-4     -GGCGGGGGGGCTCGGCCGGGCCTGGGCGGGACCAACAGCGAAGGGCAGG-GCACCAAAC
S. feltiae isolate DONR      TTGTGTGGTGTGCGGGCC--GACTCGGCGTGACCGACAGAAAAGCTTAAATACAGCGGGT
                             ....|....|....|....|....|....|....|....|....|....|....|....|
                                65        75        85        95       105       115
S. arenarium isolate IR      ------------------------------------------------------------
S. feltiae isolate KM        AAT-CTTGCTTGAGTTGAGGTCGTAAAACGAAAAGTTTCTTTCTTGCGCAATTCCTCGAC
S. feltiae isolate Kar-13    AAT-CTTGCTTGAGTTGAGGTCGTAAAACGAAAAGTTTCTTTCTTGCGCAATTCCTCAAC
S. feltiae isolate Kar-4     AAAACTTGCTTGAGTTGAGGTCGTAAAACGAAAAGTTTCTTTCTTGCGCAATTCCTCGAC
S. feltiae isolate DONR      AAT-CTTGCTTGAGTTGAGGTCGTAAAACGAAAAGTTTCTTTCTTGCGCAATTCCTCGAC
                             ....|....|....|....|....|....|....|....|....|....|....|....|
                                125       135       145       155       165       175
S. arenarium isolate IR      ------------------------------------------------------------
S. feltiae isolate KM        AAACGATACGTGTTGTGTCAAGTGATGTATTGTAAGGGCCTCTGAAAAATTCTGAAAAAT
S. feltiae isolate Kar-13    AAACGATTCGTGTTGTGTCAAGTGATGTATTGTAAGGGCCTCTGAAAAATTCTGAAAAAT
S. feltiae isolate Kar-4     AAACGATTCGTGTTGTGTCAAGTGATGTATTGTAAGGGCCTCTGAAAAATTCTGAAAAAT
S. feltiae isolate DONR      AAACGAATCGTGTTGTGTCAAGTGATGTACTGTAAGGGCCTCTGAAAAATTCTGAAAAAT
                             ....|....|....|....|....|....|....|....|....|....|....|....|
                                185       195       205       215       225       235
S. arenarium isolate IR      ------------------------------------------------------------
S. feltiae isolate KM        ATACGAACTGTTAAGTTTGTACCAATCAGTCGGCAAGAAAAACGCCGAAACACCAAAAAT
S. feltiae isolate Kar-13    ATACGAACTGTTAAGTTTGTACCAATCAGTCGGCAAGAAAAACGCCGAAACACCAAAAAT
S. feltiae isolate Kar-4     ATACGAACTGTTAAGTTTGTACCAATCAGTCGGCAAGAAAAACGCCGAAACACCAAAAAT
S. feltiae isolate DONR      ATACGAACTGTTAAGTTTGTACCAATCAGTCGGCAAGAAAAACGCCGAAACACCAAAAAT
                             ....|....|....|....|....|....|....|....|....|....|....|....|
                                245       255       265       275       285       295
S. arenarium isolate IR      ------------------------------------------------------------
S. feltiae isolate KM        ACATTGCACAGAAATAAAAAATAGCTCGTTTCGATTAGGAAACGAATTAGACAATCAAAT
S. feltiae isolate Kar-13    ACATTGCACAGAAATAAAAAATAGCTCGTTTCGATTAGGAAACGAATTAGACAATCAAAT
S. feltiae isolate Kar-4     ACATTGCACAGAAATAAAAAATAGCTCGTTTCGATTAGGAAACGAATTAGACAATCAAAT
S. feltiae isolate DONR      ACATTGCACAGAAATAAAAAATAGCTCGTTTCGATTAGGAAACGAATTAGACAATCAAAT
                             ....|....|....|....|....|....|....|....|....|....|....|....|
                                305       315       325       335       345       355
S. arenarium isolate IR      ------------------------------------------------------------
S. feltiae isolate KM        TCACATTCCAAAAAGGTACTCGATAAAAAAAACCAAATAACTAATCGAAAAAACAGTCAC
S. feltiae isolate Kar-13    TCACATTCCAAAAAGGTACTCAATAAAAAAAACCAAATAACTAATTGAAAAAACAGTCAC
S. feltiae isolate Kar-4     TCACATTCCAAAAAGGTACTCGATAAAAAAAACCAAATAACTAATCGAAAAAACAGTCAC
S. feltiae isolate DONR      TCACATTCCAAAAAGGTACTCGATAAAAAAA-CCAAATAACTAATCGAAAAAACAGTCAC
                             ....|....|....|....|....|....|....|....|....|....|....|....|
                                365       375       385       395       405       415
S. arenarium isolate IR      ------------------------------------TTGGTT---ACCAACTTT---TG
S. feltiae isolate KM        AGCAGACTGCAGGTTACGAATTAATCGACCCTCAACCAAACATACTAACAGATATAAACC
S. feltiae isolate Kar-13    AGCAGACTGCAGGTTACGAATTAATCGACCCTCAACCAAACATACTAACAGATATAAACC
S. feltiae isolate Kar-4     AGCAGACTGCAGGTTACGAATTAATCGACCCTCAACCAAACATACTAACAGATATAAACC
S. feltiae isolate DONR      AGCAGACTGCAGGTTACGAATTAATCGACCCTCAACCAAACATACTAACAGATATAAACC
                             ....|....|....|....|....|....|....|....|....|....|....|....|
                                425       435       445       455       465       475
S. arenarium isolate IR      CCCGGGT-TCAGCCGTTTTCGA----TTAACGCGGGTTCTCGCCTCAATTCACGCCCGAA
S. feltiae isolate KM        TGATAGTGCCATTTGCGTTCAAAATTTTAGCGTTCAATATGTCTGCAATTCACGCCAAAT
S. feltiae isolate Kar-13    TGATAGTGCCATTTGCGTTCAAAATTTTAGCGTTCAATATGTCTGCAATTCACGCCAAAT
S. feltiae isolate Kar-4     TGATAGTGCCATTTGCGTTCAAAATTTTAGCGTTCAATATGTCTGCAATTCACGCCAAAT
S. feltiae isolate DONR      TGATAGTGCCATTTGCGTTCAAAATTTTAGCGTTCAATATGTCTGCAATTCACGCCAAAT
                             ....|....|....|....|....|....|....|....|....|....|....|....|
                                485       495       505       515       525       535
S. arenarium isolate IR      AGCGCTCAGAGCGGCAGTCTCCGCTTTTCTSTAMRSMRCKMRSWSS-SSSGCA-TCGCMG
S. feltiae isolate KM        AACGGTTTTTGCCCCGTTTTTCATCGAACTACGAACCGAGTGATCCACCGACA--AGACT
S. feltiae isolate Kar-13    AACGGTTTTTGCCCCGTTTTTCATCGAACTACGAACCGAGTGATCCACCGACA--AGACT
S. feltiae isolate Kar-4     AACGGTTTTTGCCCCGTTTTTCATCGAACTACGAACCGAGTGATCCACCGACA--AGACT
S. feltiae isolate DONR      AACGGTTTTTGCCCCGTTTTTCATCGAACTACGAACCGAGTGATCCACCGACA--AGACT
                             ....|....|....|....|....|....|....|....|....|....|....|....|
                                545       555       565       575       585       595
S. arenarium isolate IR      CAAC--GGGCGMRGKGGRWGTWCMAARSGSRKGRRYSTWR--CTTGA-------GT-TGA
S. feltiae isolate KM        TGAT---AAATCTTTAAGTACACCTACGGCTGGTGCAGTCAAATTGATCAAAC-GT-TAA
S. feltiae isolate Kar-13    TGAT---AAATCTTTAAGTACACCTACGGCTGGTGCAGTCAAATTGATCAAAC-GT-TAA
S. feltiae isolate Kar-4     TGAT---AAATCTTTAAGTACACCTACGGCTGGTGCAGTCAAATTGATCAAAC-GT-TAA
S. feltiae isolate DONR      TGAT---AAATCTTTAAGTACACCTACGGCTGGTGCAGTCAAATTGATCAAAC-GT-TAA
                             ....|....|....|....|....|....|....|....|....|....|....|....|
                                605       615       625       635       645       655
S. arenarium isolate IR      GG-TCGTAATTCGAAAACATCGAA-------AAAATGTTGTGTTCC--AGTTGCG----C
S. feltiae isolate KM        TTGAAGTTTTTTAAGAACGGCGAGTCATAACAGACTTCTTTAATTCT-AGTCACAAGAAC
S. feltiae isolate Kar-13    TTGAAGTTTTTTAAGAACGGCGAGTCATAACAGACTTCTTTAATTCT-AGTCACAAGAAC
S. feltiae isolate Kar-4     TTGAAGTTTTTTAAGAACGGCGAGTCATAACAGACTTCTTTAATTCT-AGTCACAAGAAC
S. feltiae isolate DONR      TTGAAGTTTTTTAAGAACGGCGAGTCATAACAGACTTCTTTAATTCT-AGTCACAAGAAC
```

```
                          ....|....|....|....|....|....|....|....|....|....|....|....|
                                 665       675       685       695       705       715
S. arenarium isolate IR   CG---CGTCCACCAG-ACAGTCGACACACACGTGGCTACTACTAACTTCCA---GAAGCA
S. feltiae isolate KM     TCAAATGTCCATCACCACAGTC-ACGCTCATACAACTGCTCCAGACACCTATAGAAACCA
S. feltiae isolate Kar-13 TCAAATGTCCATCACCACAGTC-ACGCTCATACAACTGCTCCAGACACCTATAGAAACCA
S. feltiae isolate Kar-4  TCAAATGTCCATCACCACAGTC-ACGCTCATACAACTGCTCCAGACACCTATAGAAACCA
S. feltiae isolate DONR   TCAAATGTCCATCACCACAGTC-ACGCTCATACAACTGCTCCAGACACCTATAGAAACCA
                          ....|....|....|....|....|....|....|....|....|....|....|....|
                                 725       735       745       755       765       775
S. arenarium isolate IR   G-TATGTACACCAAGCT-TGGTGCA----ATTTTACT-----------------------
S. feltiae isolate KM     TTCATAGCCGTTAGTTGAGAGAATACAACGCTTTGAAACGAACAGCGAAAATTCAGCTCG
S. feltiae isolate Kar-13 TTCATAGCCGTTAGTTGAGAGAATACAACGCTTTGAAACGAACAGCGAAAATTCAGCTCG
S. feltiae isolate Kar-4  TTCATAGCCGTTAGTTGAGAGAATACAACGCTTTGAAACGAACAGCGAAAATTCAGCTCG
S. feltiae isolate DONR   TTCATAGCCGTTAGTTGAGAGAATACAACGCTTTGAAACGAACAGCGAAAATTCAGCTCG
                          ....|....|....|....|....|....|....|....|....|....|....|....|
                                 785       795       805       815       825       835
S. arenarium isolate IR   ------------------------------------------------------------
S. feltiae isolate KM     ATTCATTTGAATCCAAGTAAATGGATAAGCTCAATAATGATCCTTCCGCAGGTWMAGCCK
S. feltiae isolate Kar-13 ATTCATTTGAATCCAAGTAAATGGATAAGCTCAATAATGATCCTTCCGCAGGTTCM--CT
S. feltiae isolate Kar-4  ATTCATTTGAATCCAAGTAAATGGATAAGCTCAATAATGATCCTTCCGCAGGTTCA-CCT
S. feltiae isolate DONR   ATTCATTTGAATCCAAGTAAATGGATAAGCTCAATAATGATCCTTCCGCAGGTTCA-CCT
                          ....|....|....|....|....|....|....|....|....|....|....|....|
                                 845       855       865       875       885       895
S. arenarium isolate IR   ------------------------------------------------------------
S. feltiae isolate KM     AGCGGAGMACMTTGKTWCGACTTTTGCCCGGTT--CAAGCCGTTTCGATTAACGCGGGTT
S. feltiae isolate Kar-13 MCGGAA-MCCATTGTTACGRCTTTTGCCCGGTTACCARGCCGTTTCGATTAACGCGGGTT
S. feltiae isolate Kar-4  ACGGM--MRCWTTGYTACGACTTTTGCCCGGTT--CAAGCCGTTTCGATTAACGCGG-TT
S. feltiae isolate DONR   MCSGR--AACCWTGKTACGACTTTTGCCMGGTT--CAAGCCGTTTCGATTAACGCGG-TT
                          ....|....|....|....|....|....|....|....|....|....|....|....|
                                 905       915       925       935       945       955
S. arenarium isolate IR   ------------------------------------------------------------
S. feltiae isolate KM     CTCGCCGCAACGATCGTCCGAAAACGCTCAGAGCAGCAGTCTCCGCTTTTTCTCGAAACA
S. feltiae isolate Kar-13 CTCGCCGCAACGATCGTCCGAAAACGCTCAGAGCAGCAGTCTCCGCTTTT-CTCGAAACA
S. feltiae isolate Kar-4  CTCGCCGCAACGATCGTCCGAAAACGCTCAGAGCAGCAGTCTCCGCTTTT-CTCGAAACA
S. feltiae isolate DONR   CTCGCCGCAACRATCGTCCGAAAACGCTCAGAGCAGCAGTCTCCGCTTTT-CTCGAAACA
                          ....|....|....|....|....|....|....|....|....|....|....|....|
                                 965       975       985       995      1005      1015
S. arenarium isolate IR   ------------------------------------------------------------
S. feltiae isolate KM     ACTCAGTCCCGGGCAGCGACGGGCGGGTGTGTACAAAGGRSA-GGGACGTWWTMAAAAAA
S. feltiae isolate Kar-13 ACTCAGTCCCGGGCAGCGACGGGCGG-TGTGTACAAAGGGCAGGGAACGTAATCAAAATA
S. feltiae isolate Kar-4  ACTMAGTCCCGGGCAGCGACGGGCGG-TGTGTACAAAKGGCA-GGGASGTAGGAAWYAAA
S. feltiae isolate DONR   ACTCASTCCCKGGCAGCGACGGGCGG-TGTGTACAAARGGCAAGGGRCGTARKCAAAATA
                          ....|....|....|....|....|....|....|....|....|....|....|....|
                                1025      1035      1045      1055      1065      1075
S. arenarium isolate IR   ------------------------------------------------------------
S. feltiae isolate KM     ATTAAAAAAGGGAGGGGA-GAGG-------------------------------------
S. feltiae isolate Kar-13 AATAAAAAGGCGCGGGGACACATAATTCAAAAAACCCCCCAACCAACAAAAAAAAACAC
S. feltiae isolate Kar-4  AC--AAAGAGGGCAGGGC-ACAATATCTAAAAGAGACGACGAACAA--------------
S. feltiae isolate DONR   -CTAAAAAAAGGCGGGGA-GAGTAATTCAAAAGAGCGCGCGCACGTATGTGTGTTATCAC
                          ....|....|....|....|....|....|....|....|....|....|...
                                1085      1095      1105      1115      1125
S. arenarium isolate IR   -----------------------------------------------------
S. feltiae isolate KM     -----------------------------------------------------
S. feltiae isolate Kar-13 CACAACCAACAACAAGACGCGTCTTCGCTTGCAGCTCACGTGGACAGCTTGTC
S. feltiae isolate Kar-4  -----------------------------------------------------
S. feltiae isolate DONR   -AGACTTCTCGAGGTTGCGATAGACTACGGACGGCT-----------------
```

Fig. 2. Consensus sequence alignment of the ITS rDNA region (including partial fragments of the 18S and 28S rDNA genes) of *Steinernema* isolates.

Рис. 2. Объединённые выровненные секвенсы внутреннего транскрибируемого участка рДНК (включают в себя частино фрагменты 18S и 28S генов рДНК) штаммов *Steinernema*.

M o l e c u l a r s t u d i e s. DNA was extracted from a single first generation female or from 10–15 infective juveniles using the DNA extraction solution consisted of 17.7 μl of dd H_2O, 2 μl of 1x $MgCl_2$ TopBio™ buffer, 0.2 μl of 1 % solution of Tween–20 and 0.1 μl of proteinase K in concentration of 100 μg/ml per one sample. DNA extraction solution with nematodes was heated at 65 °C for 1 h, then at 95 °C for 10 minutes and finally centrifuged at 11600 rpm for 2 minutes.

The ITS1–5.8S–ITS2 region of the ribosomal DNA (rDNA) was amplified by PCR in a Eppendorf® MasterCycler cycling machine using the 1 μl of supernatant from reaction of DNA extraction and 11.1 μl of PCR-mix consisted of 7.25 μl of dd H_2O, 1.25 μl of 10x TopBio™ Taq-buffer, 1 μl of TopBio™ dNTP (10 mM), 0.75 μl of each primer (18S 5’ – TTG ATT ACG TCC CTG CCC TTT – 3’ (forward) and 28S 5’ – TTT CAC TCG CCG TTA CTA AGG – 3’ (reverse)) and 0.1 μl of Taq DNA-polymerase (5 U/μl) per reaction according to the cycling protocol of Vrain et al. (1992) for 26S-region, with the number of internal cycles n = 40.

Purified PCR product in volume of 10 μl was sent to a sequencing service (Macrogen Inc.). Received sequences (fig. 2) were compared with those deposited in the GenBank using BLAST 2.2.28 program (Megablast protocol of search) from Basic Local Alignment Search Tool (BLAST) of the National Centre for Biotechnology Information (NCBI) (Morgulis et al., 2008; Zhang et al., 2000).

Results and discussion

Of 196 samples, only 5 isolates of entomopathogenic nematodes were recovered from the soil studied. These isolates were designated as DONR, IR, KM, Kar–4, Kar–13 and came from Dniprovsko-Orilsky Nature Reserve, Chornomorsky Biosphere Reserve, Ukrainian Steppe Nature Reserve and Karadag Nature Reserve. The percentage of positive samples with EPN's finding reached 2.6 %.

In the sampling sites with EPN, the following soil type and plant species dominated:

1. DONR — highly humified soil; *Quercus robur* L., ***Ulmus laevis*** Pall., *Acer campestre* L., ***A. tataricum*** L., *A. platanoides* L., *Sambucus nigra* L., *Chelidonium major* L., *Urtica dioica* L., *Viola odorata* L., *Anthriscus sylvestris* (L.) Hoffm.

2. IR — dry sandy soil; ***Festuceta beckeri*** Stibick, ***Artemisieta marschallianae*** Spreng., *Phragmites australis* (Cav.) Trin. ex Steud., *Bolboschaenus maritinus* (L.) Palla, *Puccinella gigantea* Grossh.

3. KM — black soil; samples were taken beneath unusual steppe shrub of ***Rosa* sp.** — *Elytrigia* Desv., *Melica transsilvanica* Schur., *Poeta angustifoliae*, *Stipa capillata* L., *Centaurea diffusa* Lam., *Achillea millefolium* L., *Artemisia vulgaris* L., *Scabiosa* L., *Cephalaria uralensis* (Murray) Roem. & Schult., *Verbascum* L., *Echium vulgare* L., *Stipa pennata* L. = *S. joannis* Celak.

4. Kar — humified soil; ***Quercus pubescens*** Willd., *Crataegus* L., *Cotinus coggigria* Scop., *Paliurus spina-christi* Mill., *Jasminum fruticans* L., *Asphodeline taurica* (Pall.) Endl., *Artemísia* L., *Elytrigia* Desv.

Table 1. Morphometric data of infective juveniles of *Steinernema* strains

Таблица 1. Морфометрические параметры ивазионных личинок штамов *Steinernema*

Characters	DONR (n = 25) *S. feltiae*	KM (n = 25) *S. feltiae*	IR (n = 25) *S. arenarium*	Kar–4 (n = 25) *S. feltiae*	Kar–13 (n = 25) *S. feltiae*
L	706.5 ± 39.2 (627.2–789.6)	866.7 ± 18.6 (840.0–896.0)	924.2 ± 40.6 (851.2–985.6)	699.8 ± 37.2 (632.8–784)	851.4 ± 47.6 (761.6–957.6)
W	30.2 ± 2.0 (26.4–34.1)	27.2 ± 1.3 (24.8–31.0)	40 ± 8.4 (27.9–54.3)	33.3 ± 1.9 (29.5–35.7)	31.62 ± 2.8 (26.4–38.8)
T	77.8 ± 3.6 (71.3–86.8)	85.1 ± 5.1 (77.5–99.2)	75.9 ± 5.2 (62.0–83.7)	77.2 ± 3.0 (69.8–83.7)	84.5 ± 3.5 (77.5–89.9)
ES	113.1 ± 8.5 (100.8–130.2)	130.3 ± 3.6 (124.0–139.5)	130.6 ± 8.5 (114.7–147.3)	110.5 ± 4.1 (103.9–117.8)	126.2 ± 4.6 (119.4–133.3)
EP	52.5 ± 3.7 (46.5–60.5)	62.2 ± 2.7 (57.4–66.7)	76.3 ± 3.1 (71.3–82.2)	51.2 ± 3.9 (43.4–62)	60.9 ± 5.3 (54.3–77.5)
NR	91.4 ± 7.4 (77.5–108.5	91.8 ± 4.1 (85.0–100.8)	98.4 ± 4.9 (85.0–100.8)	90.1 ± 4.9 (80.6–103.9)	96.7 ± 3.4 (89.9–102.3)
D, %	46.9 ± 4.1 (39.3–54.2)	47.7 ± 2.2 (42.2–51.8)	58.6 ± 4.1 (52.2–68.9)	46.3 ± 3.9 (38.9–58.0)	48.3 ± 3.9 (42.7–61.0)
E, %	67.8 ± 6.2 (58.8–83.0)	73.2 ± 4.1 (66.1–82.7)	102.1 ± 9.2 (90.4–130.0)	66.4 ± 5.1 (58.3–83.3)	72.3 ± 6.4 (62.5–87.7)
a	23.5 ± 1.9 (19.3–26.5)	31.9 ± 1.6 (28.9–36.1)	24.1 ± 5.4 (17.7–35.0)	21.1 ± 1.4 (19.0–24.1)	27.1 ± 2.8 (23.0–34.6)
b	6.3 ± 0.5 (4.8–7.0)	6.7 ± 0.2 (6.3–7.0)	7.1 ± 0.5 (6.0–8.4)	6.3 ± 0.4 (5.6–7.0)	6.8 ± 0.4 (6.0–7.4)
c	9.1 ± 0.7 (8.1–10.4)	10.2 ± 0.6 (8.8–11.6)	12.2 ± 1.2 (10.8–15.5)	9.1 ± 0.5 (8.5–10.5)	10.1 ± 0.5 (9.3–11.1)
H	36.8 ± 0.9 (35.2–37.6)	38.8 ± 2.2 (34.9–41.2)	34.0 ± 0.8 (33.3–35.0)	32.8 ± 2.2 (29.6–35.9)	31.1 ± 1.8 (29.5–34.5)

Note. All measurements are in micrometer and in the form: Mean ± SD (min–max). L — body length; W — body diameter; T — tail length; ES — distance from anterior end to end of pharynx; EP — distance from anterior end to excretory pore; NR — distance from anterior end to nerve ring; D = EP/ ES *100 %; E = EP/ T*100 %; a = L/W; b = L/ES; c = L/T ; H — hyaline layer.

Table 2. Morphometric data of first and second generation males of *Steinernema* strains

Таблица 2. Морфометрические параметры самцов первого и второго поколения штаммов *Steinernema*

Characters	DONR (n = 8) M2 *S. feltiae*	KM (n = 11) M2 *S. feltiae*	IR (n = 5) M1 *S. arenarium*	Kar–4	Kar–13 (n = 12) M2 *S. feltiae*
L	855.4 ± 43.7 (812.0–935.2)	901.1 ± 72.7 (750.0–1019.2)	1353.0 ± 119.5 (1232.0–1495.2)	—	864.3 ± 111.5 (694.4–1064)
W	54.5 ± 7.0 (49.6–68.2)	71.4 ± 14.8 (56.0–100.8)	89.9 ± 13.0 (77.5–108.5)	—	62.5 ± 14.3 (39.2–84)
T	24.6 ± 2.9 (21.7–31.0)	26.1 ± 3.2 (20.2–31.0)	25.4 ± 3.6 (21.7–31.0)	—	20.5 ± 2.5 (18.6–24.8)
ES	125.7 ± 4.6 (117.8–131.75)	134.9 ± 7.7 (124.0–145.7)	199.0 ± 8.2 (189.1–207.7)	—	160.3 ± 10.3 (139.5–173.6)
EP	59.6 ± 12.7 (43.4–77.5)	—	76 ± 12.1 (62–83.7)	—	69.0 ± 9.5 (54.3–82.2)
NR	—	—	151.1 ± 12.0 (139.5–164.3)	—	122.2 ± 9.5 (103.9–136.4)
D%	47.4 ± 8.9 (35.0–58.8)	—	38.4 ± 6.7 (31.0–44.3)	—	43.1 ± 6.4 (34.9–53.0)
GL	41.85 ± 1.2 (40.3–43.4)	44.7 ± 1.2 (43.4–46.5)	48.7 ± 2.1 (46.5–51.2)	—	45.1 ± 4.0 (37.2–49.6)
SL	63.0 ± 2.2 (60.5–66.7)	58.6 ± 1.5 (55.8–60.5)	61.4 ± 3.6 (55.8–65.1)	—	62.3 ± 3.9 (54.3–69.8)
GL/SL	0.67 ± 0.04 (0.6–0.72)	0.76 ± 0.02 (0.74–0.81)	0.79 ± 0.05 (0.75–0.89)	—	0.73 ± 0.08 (0.59–0.86)
Muc	+	+++	—	—	++

Note. All measurements are in micrometer and in the form: Mean ± SD (min–max). L — body length; W — body diameter; T — tail length; ES — distance from anterior end to end of pharynx; EP — distance from anterior end to excretory pore; NR — distance from anterior end to nerve ring; D = EP/ES *100 %; E = EP/ T*100 %; a = L/W; b = L/ES; c = L/T ; H — hyaline layer; GL — gubernaculum length; SL — spicule length; Muc — mucron; mucron length is: “+” — 4–8 µm; “++”— 9–12 µm; “+++”— 13 µm and more).

Measurements of the nematodes collected are given in tables 1 and 2.

According to the BLAST, the steinernematid isolates Kar–4 (1043 bp), Kar–13 (1115 bp), KM (1018 bp) and DONR (1092 bp) shared sequence similarity of 98–99 % (Query coverage 84–95 %, E-value 0.00) with *S. feltiae* isolate HkEr36 (GenBank access number AB243439.1). Isolate IR (317 bp) shared sequence similarity of 93–96 % with *S. arenarium* (Artyukhovsky, 1967) Wouts, Mráček, Gerdin and Bedding, 1982, with highest identity of 96 % (Query coverage 73 %, E-value 8e–52) to strain Rjazan (GenBank access number AY230160.1). Till present *S. arenarium* has not been found in Ukraine.

Four sequences were deposited th the GenBank database: with the following accession numbers: KF939327 for *S. arenarium* isolate IR, KF939328 for *S. feltiae* isolate KM, KF939329 for *S. feltiae* isolate DONR, KF939330 for *S. feltiae* isolate Kar–4, KF939331 for *S. feltiae* isolate Kar–13 (fig. 2).

Previous studies on steinernematid fauna in Europe showed a different percentage of infected soil samples per country: from minimal 2.2 % in Scotland and 5.8 % in Finland up to maximal 53.5 % in the Czech Republic (Hominick et al., 1996; Mráček et al., 1999). Low level of EPN occurrence in North-Western Europe may be due to the cold climate and types of soil in the countries studied.

Moreover, we may explain the low level of detected EPN isolates by unfavorable period for soil sampling because the temperature is one of the most limiting factor for entomopathogenic nematodes field isolation (Georgis et al., 2006).

Some authors revealed that morphometric measurements of IJ obtained from different hosts and grown on different nutrient media are different. This could be explained by the difference in nutrients present in cultivation media (Nguyen, Smart, 1995). We assume that

the differences observed in the morphology of *S. feltiae* strains can be associated with the character and amount of the fat body in *T. molitor* larvae, as well as with temperature and humidity of the soil during the soil sampling.

We thank for the support to Ukrainian Academy of Sciences and Czech Ministry of Education, Youth and Sport and Amvis in the programme KONTAKT II, project LH 12105.

References

Erbaş, Z., Gökçe, C., Hazir, S. et al. Isolation and identification of entomopathogenic nematodes (Nematoda: Rhabditida) from the Eastern Black Sea region and their biocontrol potential against Melolontha melolontha (Coleoptera: Scarabaeidae) larvae // Turkish J. Agriculture and Forestry. — 2014.— **38**. — P. 187–197.

Georgis, R., Koppenhofer, A. M., Lacey, L. A. et al. Successes and failures in the use of parasitic nematodes for pest control // Biological Control. — 2006. — **38**, 1. — P. 103–123.

Hartelt, K., Wurst, E., Collatz, J. et al. Biological control of the tick Ixodes ricinus with entomopathogenic fungi and nematodes: Preliminary results from laboratory experiments // International J. Medical Microbiology. — 2008. — **298**. — Suppl. 1. — P. 314–320.

Hominick, W. M. Entomopathogenic rhabditid nematodes and pest control // Parasitology Today. — 1990. — **6**, N 5. — P. 148–152.

Hominick, W. M., Reid, A. P., Bohan, D. A., Briscoe, B. R. Entomopathogenic nematodes: biodiversity, geographical distribution and the convention on biological diversity // Biocontrol Science and Technology. — 1996. — **6**. — P. 317–331.

Kiryanova, E. S., Puchkova, L. V. A new parasite of the beet weevil Neoaplectana bothynoderi Kirjanova et Putschkova, sp. n. (Nematodes) // Trudy zoologicheskogo institute AN SSSR. — 1955. — **18**. — P. 53–62. — Russian : *Кирьянова Е. С., Пучкова Л. В.* Новый паразит свекловичного долгоносика Neoaplectana bothynoderi Kirjanova et Putschkova, sp. n. (Nematodes).

Laznik, Ž., Tóth, T., Lakatos, T. et al. Control of the Colorado potato beetle (Leptinotarsa decemlineata [Say]) on potato under field conditions: a comparison of the efficacy of foliar application of two strains of Steinernema feltiae (Filipjev) and spraying with thiametoxam // J. Plant Diseases and Protection. — 2010. — **117**, N 3. — P. 129–135.

Laznik, Ž., Žnidarčič, D., Trdan, S. Control of Trialeurodes vaporariorum (Westwood) adults on glasshouse-grown cucumbers in four different growth substrates: an efficacy comparison of foliar application of Steinernema feltiae (Filipjev) and spraying with thiamethoxam // Turkish J. Agriculture and Forestry. — **35**, N 6. — 2011. — P. 631–640.

Laznik, Ž., Trdan, S. An investigation on the chemotactic responses of different entomopathogenic nematode strains to mechanically damaged root volatile compounds // Experimental Parasitology. — **134**. — 2013. — P. 349–355.

Morgulis, A., Coulouris, G., Raytselis, Y. et al. Database Indexing for Production MegaBLAST Searches // Bioinformatics. — 2008. — **24**. — P. 1757–1764.

Mráček, Z., Becvar, S., Kindlmann, P. Survey of entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) in the Czech Republic // Folia parasitologica. — 1999. — **46**. — P. 145–148.

Negrisoli, de C. R. C. B., Negrisoli, A. S., jr., Bernardi, D., Garcia, S. M. Activity of eight strains of entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) against five stored product pests // Experimental Parasitology. — 2013. — **134**. —P. 384–388.

Nguyen, K. B., Hunt, D. J. Entomopathogenic nematodes: Systematics, Phylogeny and Bacterial symbionts // Nematology Monographs and Perspectives / Eds D. J. Hunt, R. N. Perry. — Leiden ; Boston : Brill, 2007. — P. 816.

Nguyen, K. B., Smart, G. C., jr. Morphometrics of infective juveniles of Steinernema spp. and Heterorhabditis bacteriophora (Nemata: Rhabditida) // J. Nematology. — 1995. — **27**. — P. 206–212.

Poinar, G. O. Description and biology of a new insect parasitic rhabditoid, Heterorhabditis bacteriophora n. gen., n. sp. (Rhabditida; Heterorhabditidae n. fam.) // Nematologica. — 1976. — **21**, N 4. — P. 463–470.

Seinhors, J. W. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin // Nematologica. — 1959. — **4**. — P. 67–69.

Sicard, M., Raimond, M., Prats, O. et al. Pathogenic effect of entomopathogenic nematode–bacterium complexes on terrestrial isopods // J. Invertebrate Pathology. — 2008. — **99**. — P. 20–27.

Steiner, G. Aplectana kraussei n. sp., eine in der Blattwespe Lyda sp. parasitierende Nematodenform, nebst Bemerkungen über das Seitenorgan der parasitischen Nematoden // Zentralblatt für Bakteriologie, Parasitenkunde und Infectionskrankheiten und Hygiene. — 1923. — **59**. — S. 14–18.

Veremchuk, G. V. A new species of entomopathogenic nematodes of the genus Neoaplectana (Rhabditida: Steinernematidae) // Parazitologiia. — 1969. — **3**, N 3. — P. 249–252. — Russian : *Веремчук Г. В.* Новый вид энтомопатогенных нематод рода Neoaplectana (Rhabditida: Steinernematidae).

Vrain, T. C., Wakarchuk, D. A., Levesque, A. C., Hamilton, R. I. Intraspecific rDNA restriction fragment lenght polymorphismis in the Xiphinema americanum group // Fundamental and Applied Nematology. — 1992. — **15**. — P. 563–574.

White, G. F. A Method for Obtaining Infective Nematode Larvae from Cultures // Science. — 1927. — **66**, N 1709. — P. 302–303.

Yan, X., Moens, M., Han, R. et al. Effects of selected insecticides on osmotically treated entomopathogenic nematodes // J. Plant Diseases and Protection. — 2012. — **119**. — P. 152–158.

Zhang, Z., Schwartz, S., Wagner, L., Miller, W. A greedy algorithm for aligning DNA sequences // J. Computational Biology. — 2000. — **7**, N 1–2. — P. 203–214.

Received 24 December 2013
Accepted 8 April 2014